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Monitoring the Structural Change of Bovine Myosin by Heating : The Comparison of Relative Fluorescence Intensity (RFI), Turbidity with Polyclonal Antibodies-based Ci-ELISA

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Thermal denaturation of myosin molecules affects to quality of comminuted (frankfurter type) and restructured meat products. And, strutural change of this protein highly relates to emulsion capacity of hydrophobic residues with fat granules. To monitor the conformational changes of bovine muscle myosin molecules by heating, we immunized rabbits with myosin whole molecules (MWM) for producing polyclonal antibodies (Abs)-based Immunoglobulin G (IgG), and established condition of Ci-ELISA RFI at 380 nm and 475 nm of exitation and emmision wavelength, respectively, turbidity at 320 nm, and immunoreactivity were measured for the myosin solution (1 mg/ml) isothermally treated from 30 to 90℃ for 10 minutes and gradient heated (1℃/mun) at the range from 30 to 90℃. No significant differences were appeared between the two heating treatments ($p>0.05$). Immunoreactivity of IgG with antigen (Ag) heated at designed temperature suffered conformational changes into two steps: the first was stage of increase of reaction of Ab with Ag at initial heating range of 40 to 55℃ (initial range), the other was stage of sharp decrease of the reaction above 55℃ to 80℃. Binding activity with anti-MWM IgG was increased from 40 to 55℃, and two peaks were appreared at 45 and 55℃ ($p<0.05$). RFI did rapidly increase at initial range, and turbidity did above 60℃ (later range) remarkably. At initial range, myosin molecules were unfolded, hydrophobic residues might be exposed to external, this can be explain by our hypothesis, which is denaturation of myosin molecules by mild heating (initial range) appeare as exposure of epitopes in Ag and immunoreactivity become more than native state. However, small range of decrease of the reactivity were at the higher heating (above 60 ℃, later range). At 80℃, turbidity had maximum rate and immunoreactivity did minimum. Rapidly processed turbidity at later range can be explicated as some conformational changes of myosin molecules developed for internal gellation of the proteins than intrachanges of molecules themselves. We concluded that the thermal change of myosin molecules could be monitored by ELISA method, and we could understand heat-dynamic changes of structure of this protein.

Key words : **bovine myosin, thermal denaturation, Ci-ELISA, relative fluorescence intensity, and turbidity**